2.0 BACKGROUND AND RATIONALE

2.1 Humans are able to generate an immune response to potential tumor relevant antigens following immunization with tumor vaccines.

There is a long history of clinical trials using various tumor preparations or extracts to immunize cancer patients. In general, these have represented rather crude attempts at active specific immunotherapy with poorly defined immunogens and less than optimal monitoring assays. These problems reflected the "state of the art" in human tumor immunology and not a shortcoming of the clinical investigators. Despite these problems, some patients had clinical evidence of antitumor effects. More recently, several vaccine trials have provided more definitive evidence of vaccine-induced antitumor responses, Several examples involve malignant melanoma studies (1,2,3). The studies by Mastrangelo's group (1,4) have utilized autologous, enzyme disassociated, irradiated tumor cells plus BCG to several sites three days following bolus cytoxan. Immunizations were repeated Q4 weeks with observed regression of metastatic tumor sites and evidence of delayed hypersensitivity skin test reactivity to autologous tumor cells. Mitchell's group (2,5) has immunized metastatic melanoma patients with allogeneic melanoma cell line lysates in DETOX (adjuvant) given weekly tunes 4 and week 6. Patients developed evidence for delayed hypersensitivity to the immunizing lysate and cytolytic T cell precursors to one of the two original allogeneic melanoma cell lines they used "cold inhibition" studies to address issues of specificity. Objective tumor responses were reported in 4/25 (2) and 5/17 patients (5). Bystryn's group (3,6,7) has immunized low tumor burden melanoma patients with a vaccine prepared from material shed into the supernate of

four different allogeneic melanoma ceil lines plus alum (adjuvant) with evidence for cellular (6) and humoral response (7) to components of the vaccine and some degree of efficacy (3).

More pertinent to this proposal, similar observations have been made in colon cancer patients (8,9,10). Hollingshead (8) reported that low tumor burden colon cancer patients immunized with a partially purified "tumor-associated antigen" preparation derived from allogeneic colon cancer sonicated membrane extracts mixed with complete Freund adjuvant and administered at multiple sites monthly times 3 could induce an immune response as determined by delayed hypersensitivity skin tests and migration inhibition assays to the "tumor-associated antigens" Hoover and Hanna (9,11,12) utilized a similar group of low tumor burden (Dukes' B and C) colon cancer patients who received autologous, enzyme disassociated, live, irradiated tumor cells (10^7) mixed with BCG weekly times 2 and tumor cells alone week 3. This randomized trial provided evidence for delayed hypersensitivity skin tests (12) to autologous tumor cells (versus normal mucosal cells) and a reduced relapse rate (9,11). A very interesting and provocative recent report by the Memorial Sloan Kettering group (10) utilized vaccine composed of a partially desialylated ovine submaxillary gland mucin alone (n=6), with DETOX (n=8) or with BCG (n=6) in colorectal cancer patients with low tumor burden (Dukes' B, C and D with no evidence of tumor). This preparation contains TN and sTN antigens expressed on various adenocarcinomas. The sTN is the epitope for B72.3 and CC49 monoclonal antibodies. This trial reported human antibody responses (IgM and IgG) in patients receiving the mucin plus adjuvant and not in the mucin alone group. All patients received pretherapy with cytoxan. Only 1 patient developed a positive delayed hypersensitivity skin test.

There are numerous other trials with these and other tumor types which provide similar observations. Overall, it seems reasonable to say that some patients receiving active specific therapy with tumor vaccines have had evidence of immune responses and occasional tumor regressions or other evidence of efficacy. However, the antigens involved are ill defined, measures of immune response are primarily skin tests and some antibody response data Methodology to evaluate T cell responses which are so prominent in animal model systems of active specific immunotherapy (13,14) is seldom included or not possible.

2.2 CEA represents a reasonable tumor relevant antigen for a tumor vaccine.

Carcinoembryonic antigen (CEA) is probably the most extensively characterized tumor-associated antigen in man (15,16). The CEA gene family belongs to the immunoglobulin super gene family and resides on the long arm of chromosome 19. It was originally thought to be present only in cancers and fetal gut but subsequently was found in small amounts in normal colon mucosa. Other members of this family which share significant homology with CEA (e.g., nonspecific crossreacting antigen or NCA) are found in other normal tissues (16,17). A large number of monoclonal antibodies to CEA and other family members have allowed extensive epitope mapping which define CEA specific epitopes (antigenic sites) as well as crossreactive epitopes and epitopes specific for other family members (18). Recently, Shively's group (19) has expressed the individual CEA domains in HeLa cells for epitope mapping purposes.

It is clear that CEA can function as an effective tumor vaccine target in an animal model of CEA expressing murine colon carcinoma line (20). Vaccination with rV-CEA produced both humoral and cellular immune responses with resultant in vivo antitumor effects. It is pertinent that mice have the CEA gene family of molecules which share 50-60% homology with human CEA (16) and yet the mice suffered no toxic effects. This serum tumor marker is widely used in clinical medicine and CEA is expressed in > 90% of colorectal tumors in man (also found in other tumor types like breast and non-small cell lung cancer). The small amounts of this 180 Kd glycoprotein in normal tissue supports the view that humans have tolerance to it. However, its immunogenicity in man is controversial with reports suggesting that colon and breast cancer patients have antibody to CEA (21,22) while other reports deny the presence of specific antibody (23,24). A recent report by Foon's group (25) demonstrated the development of antibody and T cell immunity to CEA developing as a result of immunization with a CEA anti-id vaccine. The presence of small amounts of CEA or other proteins within the CEA family in colonic mucosa and the biliary tract raises the possibility of autoimmune damage to these organ systems precipitated by immunization to CEA However, we (ref. 26 and section 2.6) and others (25,27) have observed elicitation of immune responses to CEA in patients with rV-CEA (180 Kd) or a CEA anti-id vaccine without evidence of autoimmune toxicity. The issues of tolerance, crossreacting antigens and immunogenicity will be serious concerns for this and almost any human tumor relevant antigen. From a practical perspective, these considerations have been weighed and an appropriate CEA vaccine produced under GMP conditions to allow generation of data relevant to these issues.

2.3 Vaccinia virus represents an advantageous vector for prospective tumor vaccines.

Flexner and Moss (28,29) have provided extensive reviews on the use of vaccinia virus as a live vector for carrying cloned genes. From an immunologic viewpoint, it has many advantages. This pox virus infects cells at the site of inoculation with replication in the cytoplasm. Thus, its gene products as well as appropriate inserted genes (e.g., CEA) are synthesized within autologous human cells with surface expression as well as normal peptide-class I-MHC formation and presentation on cell surfaces (for CD8 T cell recognition and response). The dendritic cells of the dermis are thought to be the most efficient antigen presenting cells, promoting T cell recognition, proliferation and lymphokine release. Animals and man normally react to recombinant vaccinia constructs with strong and prolonged humoral and cellular immune responses which have produced effective *in vivo* immunity to a variety of infectious agents (CMV, herpes, influenza, VSV, etc.) and neoplasms (in animal models). A great deal of vaccine research in AIDS is utilizing vaccinia vectors as recently reviewed by Hu (30,31).

From a practical standpoint, its economy of manufacture, standardization, stability in freeze-dried form and simplicity of administration by skin scratch or jet gun are important advantages. Its safety record with worldwide use is impressive and responsible for the eradication of smallpox.

Prior vaccination with vaccinia (most of U.S. population in colon cancer age range) limits the degree of local infection on revaccination and potentially could restrict the immune response to

the candidate tumor antigen (see **Discussion** in 28.31). However, several examples of immune response to HIV env in previously vaccinated individuals have been documented

(30,31,32,33). In previously vaccinated subjects, the acute local inflammation (viral infection) is substantially less than in naive recipients. In general, the size of the local lesion appears to correlate with the degree of immune response (reviewed in 28.29). For example, the study by Cooney (31) using rV-HIV env in normal volunteers demonstrated that previously vaccinated patients (majority > 15 years previous) had mild-moderate local inflammation of similar degree at 10^8 or 10^9 pfu/ml "doses" including similar immune responses. A small number of non-vaccinated volunteers received a "dose" of 10^6 and 10^7 pfu/ml with larger local sites of inflammation (infection) and greater immune responses than the patients receiving 10^8 or 10^9 pfu/ml doses. Thus, the dose of recombinant antigen is related to local viral replication in cells and not to the dose of vaccinia applied to the scarified site (at least in previously vaccinated recipients).

These recombinant vaccinia vaccines have many advantages that make them excellent candidates for human tumor vaccine trials.